J. Physiol. (1985), **365**, *pp.* 121–130
With 4 text-figures
Printed in Great Britain

CANINE BOMBESIN-LIKE GASTRIN RELEASING PEPTIDES STIMULATE GASTRIN RELEASE AND ACID SECRETION IN THE DOG

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(*Received 20 November 1984*)

SUMMARY

1. The synthetic mammalian bombesin-like peptides, canine gastrin releasing peptide 27, 23 and 10, and porcine gastrin releasing peptide 27 were compared with amphibian bombesin 14 and 10 during intravenous infusions into six conscious dogs with chronic gastric cannulae.

2. Gastrin and gastrin releasing peptide were measured in peripherally sampled venous blood by radioimmunoassay and gastric acid secretions were collected.

3. All forms of gastrin releasing peptide stimulated gastrin release and gastric acid secretion in a dose-dependent manner. The larger canine and porcine peptides were more potent than the decapeptide. Bombesin 14 was more potent than bombesin 10.

4. A rise in the venous concentration of immunoreactive gastrin releasing peptide of only 20 fmol ml^{-1} stimulated gastrin release to about 50% of maximal.

5. Gastrin releasing peptide 10 was cleared from the circulation three times faster than the larger forms and this may account for the apparent differences in potency.

INTRODUCTION

Gastrin releasing peptide is the mammalian equivalent of bombesin, a tetradecapeptide from amphibian skin (Anastasi, Erspamer & Bucci, 1972). Gastrin releasing peptide was first isolated from the fundic mucosa of the porcine stomach as a peptide of twenty-seven amino acids that shared the carboxyl-terminal heptapeptide with bombesin (McDonald, Nilsson, Vagne, Ghatei, Bloom & Mutt, 1978; McDonald, Jornvall, Nilsson, Vagne, Ghatei, Bloom & Mutt, 1979). By use of immunocytochemistry gastrin releasing peptide has been localized to nerve fibres of the myenteric

The experiments were carried out at the Center for Ulcer Research and Education.

plexus and the mucosa in the stomach and intestine (Dockray, Vaillant & Walsh, 1979), where the peptide is regarded as a putative neurotransmitter. A physiological role has yet to be ascribed to gastrin releasing peptide although it exhibits a wide range of pharmacological actions, the most well known of which is the stimulation of gastrin release and gastric acid secretion (Bertaccini, Erspamer, Melchiorri & Sopranzi, 1974; McDonald *et al.* 1978, 1979; McDonald, Ghatei, Bloom, Adrian, Mochizuki, Yanaihara & Yanaihara, 1983*a*; McDonald, Ghatei, Bloom, Track, Radzvik, Dupré & Mutt, 1983*b*). The minimal sequence of the peptide required for full stimulation of gastrin release or smooth muscle motility appears to be the carboxyl-terminal nonapeptide sequence (Erspamer & Melchiorri, 1973; Broccardo, Falconieri, Erspamer, Melchiorri, Negri & de Castiglione, 1975).

TABLE 1. The amino acid sequences of gastrin releasing peptide (GRP) and bombesin. The sequence of amino acids required for full biological activity is enclosed. Italicized residues represent differences from the porcine peptide

Porcine GRP27	A	P	V	*S*	*V*	G	*G*	G	T	V	L	*A*	K	M	Y	P	R	G	N	*H*	W	A	V	G	H	L	M-NH_2
Canine GRP27	A	P	V	*P*	*G*	G	*Q*	G	T	V	L	*D*	K	M	Y	P	R	G	N	*H*	W	A	V	G	H	L	M-NH_2
Canine GRP23					*G*	G	*Q*	G	T	V	L	*D*	K	M	Y	P	R	G	N	*H*	W	A	V	G	H	L	M-NH_2
Canine GRP10																		G	N	*H*	W	A	V	G	H	L	M-NH_2
Amphibian bombesin 14													*Q*	*Q*	*R*	*L*	G	N	*Q*	W	A	V	G	H	L	M-NH_2	
Amphibian bombesin 10																		G	N	*Q*	W	A	V	G	H	L	M-NH_2

Recently, three molecular variants of gastrin releasing peptide have been isolated and sequenced from the muscle of the canine small intestine (Reeve, Walsh, Chew, Clark, Hawke & Shively, 1983). These peptides, with twenty-seven, twenty-three and ten amino acids, share the carboxyl-terminal sequence of fifteen amino acids with porcine gastrin releasing peptide 27 (Table 1). The ten amino acid peptide was isolated subsequently from porcine spinal cord and given the new name 'neuromedin C' (Minamino, Kangawa & Matsuo, 1984). In the present study we have chosen to call the mammalian bombesin-like peptides gastrin releasing peptides (GRP), designating the species from which they were isolated when appropriate, as well as the number of amino acid residues; e.g. porcine GRP 27, GRP 10. In the present investigation the effects of canine and porcine gastrin releasing peptides and amphibian bombesin on gastrin release and gastric acid secretion in the conscious dog were examined.

METHODS

Surgical preparation. Mongrel dogs (male and female, 20–30 kg) were anaesthetized with sodium pentabarbitone and halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). A cannula was inserted in the gastric corpus near the antral junction of the greater curvature of the stomach and then exteriorized through the left lateral abdominal wall.

Experimentation. Experiments commenced on conscious dogs at least 30 days after surgery. Food but not water was withheld for 20 h before experimentation and the animals were accustomed to the experimental routine. The dogs were gently restrained in a sling and a polyethylene catheter was inserted into a peripheral vein of the hind leg for administration of the peptides. A similar catheter was placed into a peripheral vein of the foreleg for withdrawal of blood. The gastric cannula was unplugged, the stomach was irrigated with tap water and a tube was attached to the

cannula for collection of gastric secretions. The animals were rested for 45 min before infusions commenced. At the start of the experiment physiological saline (0·15 M) containing 0·5% bovine serum albumin was infused intravenously at 30 ml h^{-1}. Gastric secretions were collected every 15 min and after 30 min 5 ml blood was withdrawn from the peripheral vein. After a basal period of 30 min gastrin releasing peptide or bombesin were added to the infusate to give doses of 3·125, 12·5, 50, 200, 800, 3200 and 12800 pmol kg^{-1} h^{-1}. Starting with the lowest dose, the peptides were infused for 45 min periods in ascending order of doses. At the end of each 45 min period, 5 ml blood was withdrawn from a peripheral vein for assay of gastrin and gastrin releasing peptide. Gastric secretions were collected every 15 min during the experiments. The interval between experiments on individual dogs was at least 48 h.

Analysis of samples. Blood was withdrawn into chilled glass tubes containing ethylenediaminetetraacetic acid (0·125 mg ml^{-1} blood) and Trasylol (FBA Pharmaceuticals, New York, NY, U.S.A. (500 K.I.U. ml^{-1} blood). Within 10 min of withdrawal, blood samples were centrifuged (2000 *g*, 30 min, 4 °C) and plasma was stored in aliquots in glass tubes at −40 °C until assayed.

Gastrin. Gastrin was measured in plasma by radioimmunoassay using the carboxyl-terminal specific antibody 1611 (Rosenquist & Walsh, 1980).

Gastrin releasing peptide. Gastrin releasing peptide was measured in plasma by radioimmunoassay by a modification of previously described methods (Walsh & Wong, 1979). The modification involved using GRP decapeptide as a standard. Radioiodinated Tyr bombesin was used as the label. Antibody 1078, which is carboxyl-terminal directed, and thus cross-reacts fully with all forms of gastrin releasing peptide, was used. The half-maximal displacement of label binding was obtained at a concentration of decapeptide of 10·4 fmol ml^{-1}. Prior to assay the plasma samples were extracted with ethanol to precipitate plasma proteins (1 ml plasma mixed with 3·6 ml absolute ethanol). The supernatant was concentrated to a volume of 60 μl by vacuum centrifugation. Recovery of GRP 10 added to plasma averaged 65%.

Gastric acid. The total acidity of the gastric secretions was determined by titration of a measured aliquot with 0·2 M-sodium hydroxide to pH 7·0 and correction for the total volume.

Peptides. Canine gastrin releasing peptide 27, 23 and 10 were prepared by solid phase synthesis using a chemical strategy derived from the synthesis of the porcine peptide (Marki, Spiess, Taché, Brown & Rivier, 1981; Reeve *et al.* 1983). Porcine gastrin releasing peptide 27 and bombesin 10 and 14 were purchased from Peninsula Laboratories, San Carlos, CA, U.S.A. The homogeneity of the peptides used in this study was established by high pressure liquid chromatography using a reverse-phase C-18 column (5 μm pore size, 25 × 0·5 cm). Samples were loaded onto a column equilibrated in 0·1% trifluoroacetic acid and eluted by increasing concentrations of acetonitrile. The amino acid composition of all peptides was determined by amino acid analyses, in triplicate. A weighed amount of peptide was hydrolysed in constant boiling hydrochloric acid in sealed tubes under vacuum at 110 °C for 24 h. The hydrolysate was analysed using a Beckman 6300 amino acid analyser (Beckman Instruments Inc. Palo Alto, CA, U.S.A.). For infusion into animals the peptides were dissolved in physiological saline (0·15 M), containing 0·5% bovine serum albumin to prevent loss of the peptides on the infusion tubing.

Statistical analyses. The potencies of the peptides for the stimulation of gastrin release were compared to that of bombesin 14 using the jack-knife method (Elashoff, 1981). This type of analysis is loosely equivalent to computing the relative potency with data from each dog deleted in turn and then computing the mean and standard error of these estimates. The gastric acid responses were too variable for a strict statistical analysis.

RESULTS

Analysis of peptides. The peptides used in this study were analysed by high pressure liquid chromatography and by amino acid analysis. All peptides chromatographed as a single peak. The amino acid analyses agreed with the expected compositions and were consistent with the absence of contaminating peptides. However, the yields of the peptides were less than expected and indicated that each sample contained between 29 and 47% impurities which were probably salt and water. These impurities were accounted for in the calculation of the peptide doses infused into the dogs.

Gastrin release. The intravenous infusion of all of the forms of bombesin and gastrin releasing peptide stimulated the secretion of immunoreactive gastrin into the general circulation in a dose-related manner (Fig. 1 *A* and *B*). With regard to gastrin releasing peptide, the dose–response curves were parallel (Fig. 1 *A*), but the threshold doses

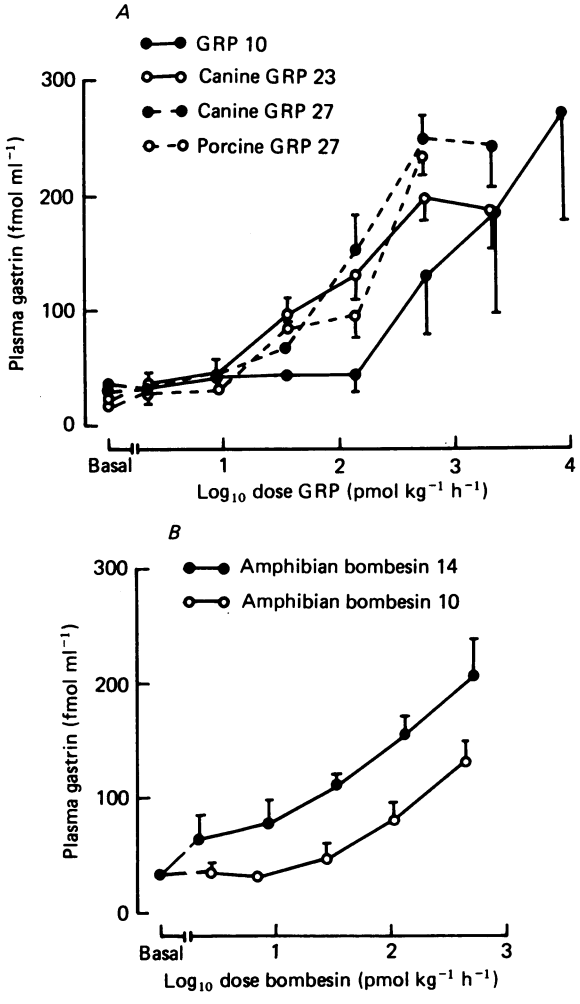

Fig. 1. The effects of intravenous infusions of canine and porcine gastrin releasing peptide (GRP) (*A*) and amphibian bombesin (*B*) on the concentration of immunoreactive gastrin measured in peripherally sampled venous blood in the conscious dog. Mean ± S.E. of mean, $n = 6$.

required to stimulate gastrin release significantly above basal values were less for the larger canine and porcine peptides than for the decapeptide. In addition, whereas the dose–response curves for the larger peptides were almost superimposable, the decapeptide was significantly less potent than canine gastrin releasing peptide 23 ($P < 0{\cdot}05$). The gastrin response to gastrin releasing peptide 27 and 23 reached a plateau at high doses of the peptides, above 800 pmol kg^{-1} h^{-1}. The dose–response

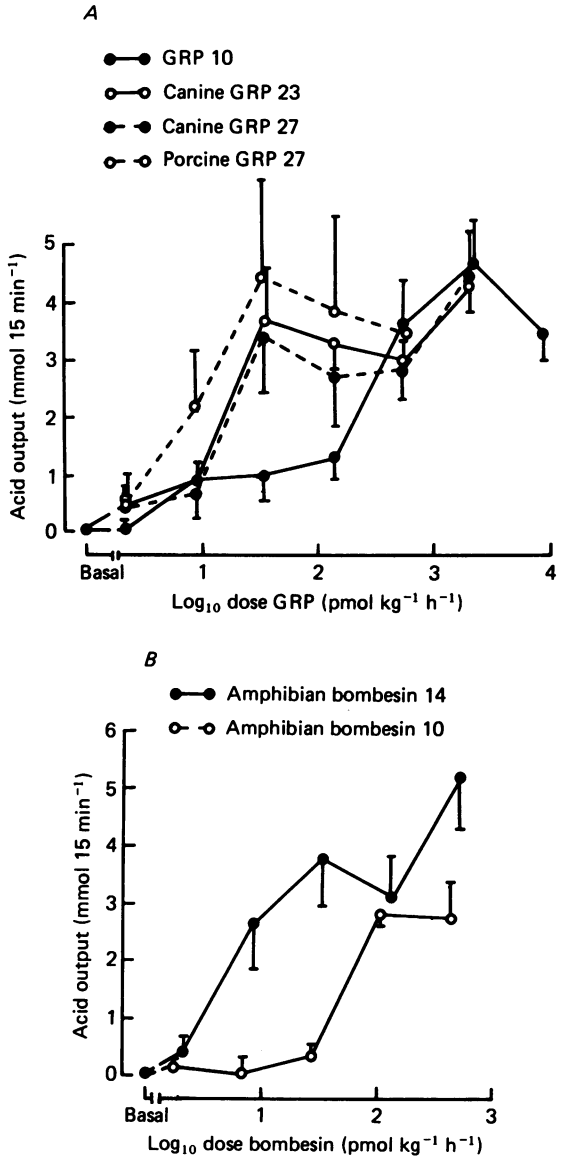

Fig. 2. The effects of intravenous infusions of gastrin releasing peptide (GRP) (*A*) and bombesin (*B*) on gastric acid secretion in the conscious dog. Mean ± S.E. of mean, $n = 6$.

curves for bombesin 10 and bombesin 14 (Fig. 1*B*) were parallel to the gastrin releasing peptide dose–response curves. The threshold dose of bombesin 14 that was required to stimulate gastrin release significantly above basal was less than that required for bombesin 10 or gastrin releasing peptide 10, 23 or 27. Bombesin 10 and gastrin releasing peptide 10 were significantly ($P < 0{\cdot}05$) less potent than bombesin 14. At lower doses, bombesin 14 appeared to be more potent than gastrin releasing peptide 23 or 27 but the dose–response curves merged together at the higher doses.

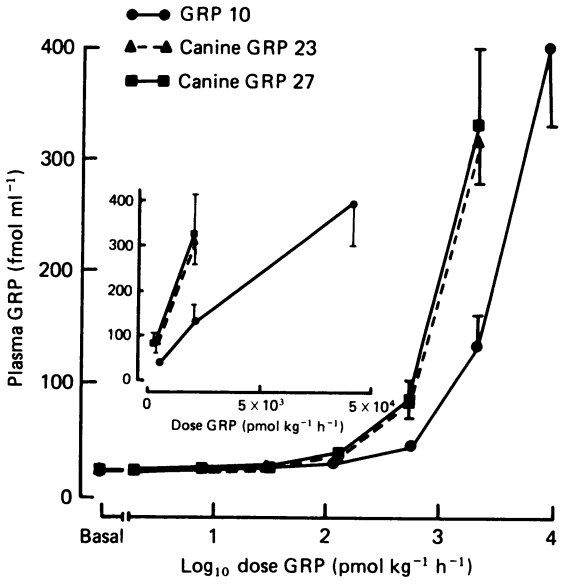

Fig. 3. The effects of intravenous infusions of gastrin releasing peptide in the conscious dog on the concentration of immunoreactive gastrin releasing peptide (GRP) in peripheral venous blood. Mean ± S.E. of mean, $n = 6$.

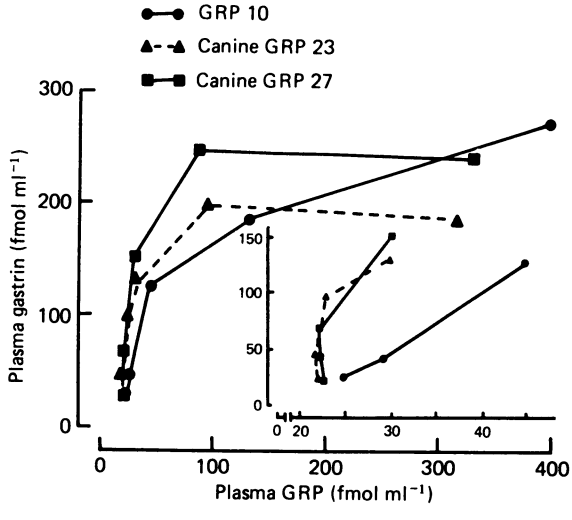

Fig. 4. The relation between the concentrations of immunoreactive gastrin releasing peptide (GRP) and gastrin in peripheral venous blood. Mean ± S.E. of mean, $n = 6$.

Acid secretion. All forms of GRP and bombesin stimulated gastric acid secretion dose dependently (Fig. 2*A* and *B*). Considering the gastrin releasing peptides, the larger canine and porcine forms appeared to be more potent than the canine decapeptide in the mid-dose range, although higher doses of the decapeptide were as capable of stimulating gastric acid secretion as the larger peptides (Fig. 2*A*). A

depression of the secretion of acid was consistently apparent at doses of porcine and canine gastrin releasing peptide 23 and 27 at 50 pmol kg^{-1} h^{-1}, and for the decapeptide at doses above 3200 pmol kg^{-1} h^{-1}. A similar depression was observed for bombesin 14 at doses more than 50 pmol kg^{-1} h^{-1}. Bombesin 14 appeared to be a stronger stimulant of acid secretion than bombesin 10 at most doses (Fig. 2*B*).

TABLE 2. The metabolic clearance rate of immunoreactive canine gastrin releasing peptide (GRP) calculated as the actual infusion rate for the highest dose of peptide divided by the change in peptide concentration in peripheral venous plasma. Values were calculated for the highest dose of each peptide that was infused. Mean ± S.E. of mean, $n = 6$

Peptide	Metabolic clearance rates (l kg^{-1} h^{-1})
Canine GRP10	28·25 ± 0·45
Canine GRP23	6·65 ± 0·45
Canine GRP27	8·45 ± 3·72

Measurement of gastrin releasing peptide in plasma. The basal concentration of immunoreactive gastrin releasing peptide measured in peripherally sampled venous plasma from the fasted dog was 22 ± 2 fmol ml^{-1} ($n = 6$). The intravenous infusion of graded doses of canine gastrin releasing peptide produced no detectable change in the circulating concentrations of immunoreactive gastrin releasing peptide until the infusion rates exceeded 200 pmol kg^{-1} h^{-1}, by which point both the release of gastrin and the secretion of gastric acid were stimulated above basal values (Fig. 3). Infusion rates of 800 and 3200 pmol kg^{-1} h^{-1} for canine gastrin releasing peptide 27 and 23 produced a higher concentration of plasma immunoreactive peptide than the same dose of the decapeptide. The relation between the plasma gastrin concentration and the circulating concentrations of canine gastrin releasing peptide is shown in Fig. 4. For both the 23 and 27 peptides the maximal gastrin response was produced by circulating concentrations of immunoreactive gastrin releasing peptide below 100 fmol ml^{-1}. Indeed, a rise of only 20 fmol ml^{-1} above basal produced about 50% of the maximal gastrin response. In separate studies, basal concentrations were similar and were not altered by atropine or by sham feeding.

Clearance rates. The clearance rates for gastrin releasing peptide 10, 23 and 27 were calculated from the higher rates of infusion as the actual infusion rate divided by the change in immunoreactive peptide measured in plasma (Table 2). Gastrin releasing peptide 23 and 27 were cleared about 3–4 times more slowly than the decapeptide.

DISCUSSION

It is well established that the amphibian peptide bombesin and its mammalian counterpart gastrin releasing peptide are potent stimulants of gastrin release and gastric acid secretion in the dog. In dogs, the intravenous infusion of bombesin 14 produces a potent stimulation of gastrin release and acid secretion (Bertaccini *et al.* 1974), and is of comparable potency to porcine gastrin releasing peptide 27 (McDonald *et al.* 1983*a*, *b*). The present study compares the effects of various molecular forms of canine and porcine gastrin releasing peptide and amphibian bombesin on the gastric responses of the conscious dog. The results show that the large and small

molecular forms of gastrin releasing peptide and bombesin powerfully stimulate both gastrin release and gastrin acid secretion in a dose-dependent fashion, but that there are some differences between the responses to certain peptides. The larger forms of gastrin releasing peptide, of twenty-seven and twenty-three amino acid residues, and bombesin 14 tended to stimulate a greater secretion of gastrin and gastric acid than the same dose of gastrin releasing peptide and bombesin decapeptide in the mid portion of the dose range (50–800 pmol kg^{-1} h^{-1}). Since all of these peptides share an almost identical biologically active carboxyl sequence of nine amino acids it is unlikely that these apparent differences in potency are due to actual differences in potency at the target cell. Indeed, the large and small forms of gastrin releasing peptide and bombesin are equally potent stimulants of contraction of isolated strips of canine gastric muscle (Mayer, Reeve, Khawaja, Chew, Elashoff, Clark & Walsh, 1984). Rather, the differences in potency are more likely to be related to differences in the clearance rates of the peptides from the general circulation. In the present investigation, the decapeptide of gastrin releasing peptide was cleared about three times faster than the twenty-three or twenty-seven residue forms and this could account for apparent differences in the potency of the various peptides. Amino terminal extensions of gastrin releasing peptide 10 may protect the biologically active portion from catabolism as it passes through the body. This is true for a number of peptides. For example, somatostatin 28 has a 5-fold longer half-life than the tetradecapeptide (Seal, Yamada, Debas, Hollinshead, Osadchey, Aponte & Walsh, 1982) and shorter forms of gastrin are more efficiently extracted by the liver than the longer peptides (Strunz, Thompson, Elashoff & Grossman, 1978). Gastrin releasing peptide 27 has a short half-life in the general circulation, with linear components of the disappearance curve giving half-lives of 1·4 and 6·6 min (Knuhtsen, Holst, Knigge, Olesen & Nielsen, 1984). The site of the clearance of gastrin releasing peptide from the general circulation is unknown. However, the neutral endopeptidase EC 3 . 4 . 24 . 11 efficiently inactivates gastrin releasing peptide 10 by cleavage of the His^8–Leu^9 bond (Bunnett, Kobayashi, Orloff, Turner, Reeve & Walsh, 1985). This membrane-bound peptidase is present in many tissues but is concentrated particularly in the kidney microvilli (Matsas, Fulcher, Kenny & Turner, 1983).

The gastric acid response to bombesin is abolished by antrectomy (Impicciatore, Debas, Walsh, Grossman & Bertaccini, 1974) and is thus mediated by the secretion of gastrin. Gastrin releasing peptide and bombesin stimulated gastric acid secretion in a dose-dependent way, and apparent differences in potency probably reflect differences in the potency with respect to gastrin release. There was a transient but consistent depression in the acid responses to higher doses of the peptides. Gastrin is not the only peptide released by gastrin releasing peptide, and the secretion of other peptides, such as somatostatin or cholecystokinin, may have been responsible for this depression in acid secretion (Martindale, Kauffman, Levin, Walsh & Yamada, 1982; Inoue, McKay, Yajima & Rayford, 1983).

Gastrin releasing peptide is localized exclusively in nerves of the mammalian alimentary tract (Dockray *et al.* 1979) but it is unclear whether it acts as a neurotransmitter or a circulating hormone. Electrical stimulation of the vagal nerve of the anaesthetized pig released peptides similar to gastrin releasing peptide 27 and

10 into the gastric venous blood (Knuhtsen *et al.* 1984). In the conscious calf gastrin releasing peptide may have a hormonal role. Stimulation of the cut end of the splanchnic nerve stimulated gastrin releasing peptide release into arterial blood and the intravenous infusion of exogenous peptide to duplicate these arterial concentrations potently stimulated insulin secretion, but gastrin was unaffected (Bloom & Edwards, 1984). The present investigation shows that very small changes in immunoreactive gastrin releasing peptide in the general circulation, of only 50 fmol ml^{-1}, can result in a maximal secretion of gastrin, but whether such changes occur under physiological conditions is unknown.

Supported by NIH grants AM 17294 and 17328, by the Veterans Administration and by M.R.C. funds. Anita Starlight kindly typed the manuscript.

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